

KJELDAHL DETERMINATION OF NITROGEN IN
REFRACTORY MATERIALS*

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Since 1883, when J. Kjeldahl (9) introduced his method for the determination of nitrogen in certain compounds pertaining to the brewing industry, many improvements have been made to extend its usefulness for the analysis of nitrogen compounds in general. Investigators have studied innumerable phases of the problem with the object of increasing the accuracy and shortening the digestion time. Even today it is a subject of much interest to analytical chemists.

In the investigation reported here, a study was made of the application of the Kjeldahl procedure to heterocyclic and other aromatic nitrogen compounds generally considered refractory. Many catalysts have been proposed and used in the Kjeldahl procedure, and it was hoped that through the study of these compounds, a better evaluation of catalysts could be made. Nicotinic acid was chosen for most of these studies, since it is a representative compound of this type, can be obtained pure, and meets most of the requirements of a primary standard. Particular attention was given to the catalysts selenium, mercuric oxide, and potassium sulfate, together with times of digestion required for the determination of nitrogen by the Kjeldahl method. Neither copper nor any of its salts were included, since in previous work at this Laboratory (13), copper had no catalytic effect when used with mercury and was inferior to mercury when the two were used separately.

The use of selenium as a catalyst was first recommended by Lauro (10, 11), but later questioned by Osborn and Wilkie (14). Patel and Sreenivasan (15) studied the relationship of selenium to the recovery of nitrogen and to the loss of nitrogen. Sandstedt (18) reported low results for total nitrogen after prolonged digestion with selenium. Davis and Wise (6), Snider and Coleman (20), Dalrymple and King (5), and others reported similar losses of nitrogen, and concluded it does not appear practicable to employ selenium catalysts because digestion time would have to be determined and also controlled in order to obtain the maximum yield of nitrogen. Bradstreet (3, 4) also found progressively lower nitrogen values with increasing quantities of selenium. On the other hand, Illarionov and Soloveva (8) stated that the catalytic action of selenium was

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proportional to the amount used. Furthermore, Davis and Wise (6) observed that selenium interfered when the amount of potassium sulfate exceeded 13 g. Shirley and Becker (19) reported that a mixture of selenium oxychloride and mercury is a satisfactory catalyst for the Kjeldahl nitrogen determination of ring-type nitrogen compounds. This was likewise reported by Belcher and Godbert (2), who found that none of the catalysts they tried proved equal in efficiency to the selenium-mercury catalyst.

In addition to having an efficient catalyst for the digestion of nitrogen compounds in the Kjeldahl method, it is important to have the proper amount of potassium sulfate. The first to recognize this was Gunning (7). Later Phelps and Daudt (16) stated that the amount of potassium sulfate and sulfuric acid in the presence of mercury determines the completeness of the hydrolysis.

Alcock (1) reported experiments which showed that a definite relationship exists between sodium sulfate, sulfuric acid, and digestion time in their effect on nitrogen recovery.

PROCEDURE

REAGENTS

1. *Catalysts*.—Metallic selenium powder and mercuric oxide.
2. *Sodium hydroxide-sodium thiosulfate soln.*—Mix 100 ml of 50% sodium hydroxide and 25 ml of 8% sodium thiosulfate soln.
3. *Indicator*.—Mix 400 ml of 0.1% methyl red dissolved in 95% ethanol with 100 ml of 0.1% methylene blue also dissolved in 95% ethanol.
4. *Boric acid*.—Dissolve 4 g of boric acid in 100 ml of distilled water.
5. *Standard acid*.—0.1 *N* hydrochloric acid.

The Kjeldahl method followed differed little from the one in common use. In addition to the sample, the digestion mixture consisted of sulfuric acid, potassium sulfate and 2 catalysts. A sample containing at least 30 mg. of nitrogen was used so that 20 ml. or more of standard 0.1 *N* acid would be required in the titration. In all cases, the digestion was made in a 650-ml. Kjeldahl flask with 25 ml. of sulfuric acid, to which was added different amounts of potassium sulfate, mercuric oxide, and selenium. The mixture was digested for 1 to 8 hours with full boiling; chips were used to maintain uniform ebullition. The time of boiling had no relation to the time required to clear the solution.

The digest was diluted with 250 ml. of distilled water, and made alkaline with sodium hydroxide-sodium thiosulfate solution; 5 g. of 20-mesh zinc granules were then added. The distilling apparatus used a modified connecting bulb (21) and a 500-ml. wide-mouth Erlenmeyer receiving flask sealed with a trap (17). The condenser tube extended below the surface of the 100 ml. of 4% boric acid solution used as the trapping liquid.

RESULTS

Figure 1 shows the effect on the recovery of nitrogen in nicotinic acid of varying amounts of selenium with three different weights of potassium sulfate, with and without 0.4 g. of mercuric oxide. A digestion time of 6 hours was used.

These results indicate that to obtain theoretical recoveries of nitrogen with 5.5 g. of potassium sulfate, the optimum amount of selenium was between 0.25 g. and 0.40 g., whereas with 11.0 or 16.5 g. of potassium sulfate, less than 0.015 g. of selenium could be used. In the absence of

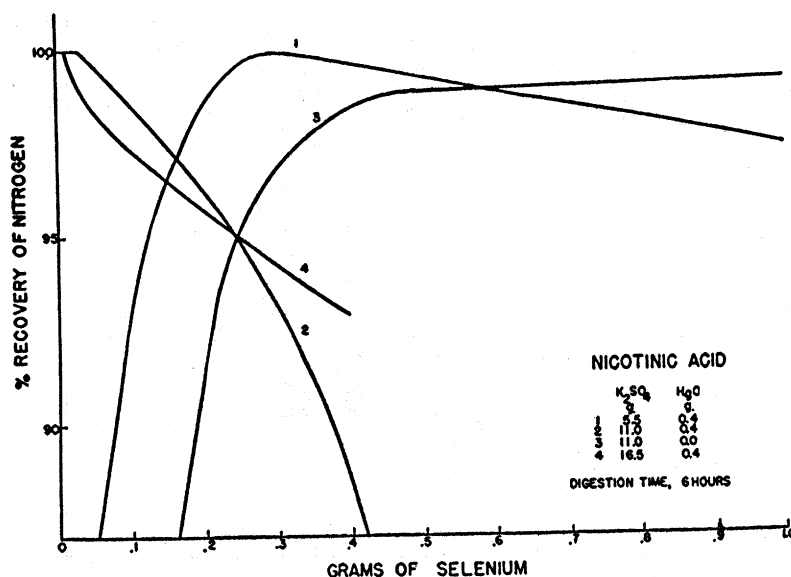


FIG. 1.—Recovery of nitrogen as affected by varying amounts of selenium with different amounts of potassium sulfate.

mercury, as much as 1.50 g. of selenium to 11 g. of potassium sulfate gave incomplete recovery.

These results indicated that some optimum ratio of selenium to potassium sulfate might be found. To determine this, studies were made with different amounts of potassium sulfate and 0.3, 0.03 and 0.015 g. of selenium. Figure 2 shows that the optimum amount of selenium decreases as the amount of potassium sulfate increases. However, with a prolonged digestion time, in this case 6 hours, it was easily possible to exceed the optimum amount of potassium sulfate to be used with 0.3 g. or 0.03 g. of selenium, with a resultant loss of nitrogen. The long digestion period of 6 hours was chosen so that any loss of nitrogen would be recognized as such and not confused with incomplete digestion. The use of 0.015 g.

of selenium had little influence, since the recovery of nitrogen was essentially the same as when no selenium was used. Three-hundredths gram of selenium gave maximum recoveries when used with 11 g. of potassium sulfate but caused a loss of nitrogen with larger amounts. This increasing loss of nitrogen with increasing amounts of potassium sulfate, as would be expected, was more pronounced when larger amounts (0.3 g.) of selenium were used.

From the preceding experiments, it appeared desirable to establish if, when large amounts of selenium (0.30 g.) and varying amounts of potas-

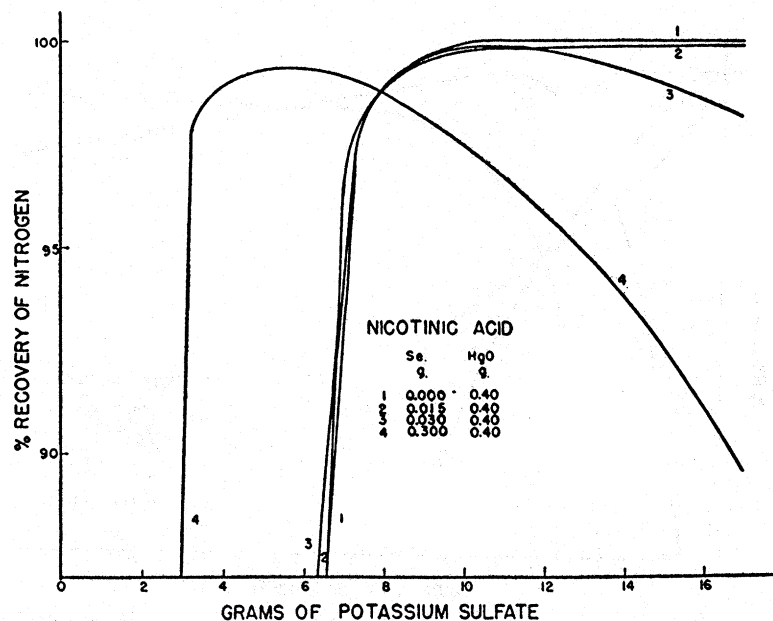


FIG. 2.—Recovery of nitrogen as affected by varying amounts of potassium sulfate with different amounts of selenium.

sium sulfate are used, there is some specific digestion time in which theoretical or maximum recoveries of nitrogen can be obtained. Figure 3 shows that for mixtures containing 0.3 g. of selenium and 5.5 g. of potassium sulfate, 99.8% of the nitrogen of nicotinic acid is recovered after 6 hours digestion, and that there is no apparent loss of nitrogen after 7 and 8 hours. With larger amounts of potassium sulfate, 11 to 15 g., the maximum but less than theoretical recovery of nitrogen occurs with a digestion time of between 2 and 4 hours, and a loss of nitrogen occurs with longer periods. Since the maximum recovery with 11 or more g. of potassium sulfate and 0.30 g. of selenium was less than theoretical, and since the recovery diminished with longer digestion periods, it was decided that

for all future experiments a smaller amount (0.03 g.) of selenium should be used.

It was indicated in Figure 2 that theoretical recoveries of nitrogen could be had without the use of selenium, but there was no indication as to the effect of digestion time on the recovery. A series of determinations was therefore made with varying amounts of potassium sulfate, and 0.4 g. of mercuric oxide with digestion times of 1 to 8 hours. A parallel experiment was made in which 0.03 g. of selenium was added to the digestion

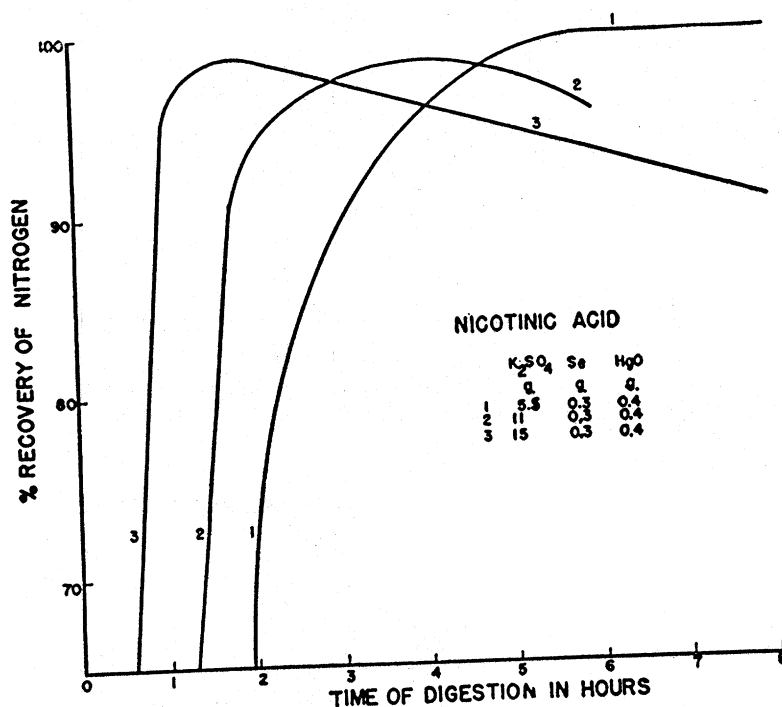


FIG. 3.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of selenium.

mixture. The results of these two experiments are shown in Figures 4 and 5, respectively.

A comparison of Figures 4 and 5 shows that the best digestion mixture contained 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and no selenium, since it required the shortest digestion period (2 hours) for complete recovery of nitrogen and prolonged digestion caused no loss of nitrogen. With low concentration of sulfate salts, selenium caused a somewhat more rapid rate of nitrogen recovery, whereas with higher concentrations the reverse was true.

In all the preceding experiments, the amount of mercuric oxide, 0.4

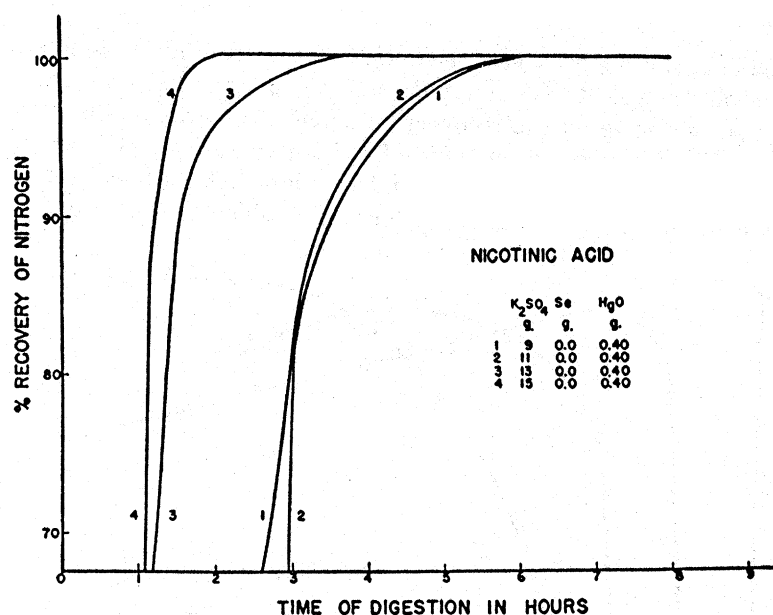


FIG. 4.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate, without selenium.

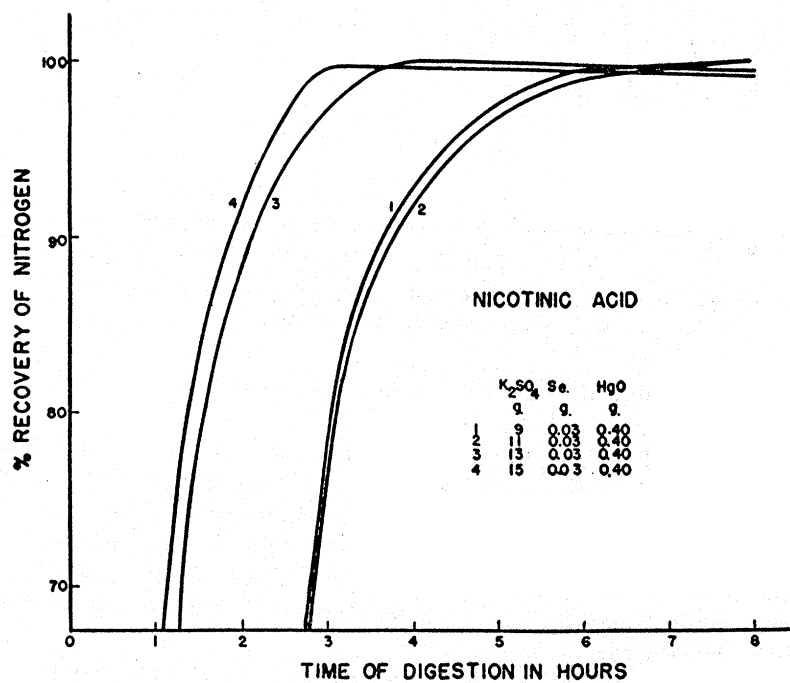


FIG. 5.—Recovery of nitrogen as affected by varying the time of digestion with different amounts of potassium sulfate with selenium in the mixture.

g., was chosen arbitrarily. Experiments were therefore made, as shown in Figure 6, to determine the effect of varying the amount of mercuric oxide. For digestion mixtures containing 15 g. of potassium sulfate and *no mercuric oxide*, less than 55% of the nitrogen was recovered with a 3-hour digestion. The amount of mercuric oxide required with this quantity of potassium sulfate to give complete recovery of nitrogen was not critical, since as little as 0.1 g. or as much as 1 g. gave equally satisfactory results.

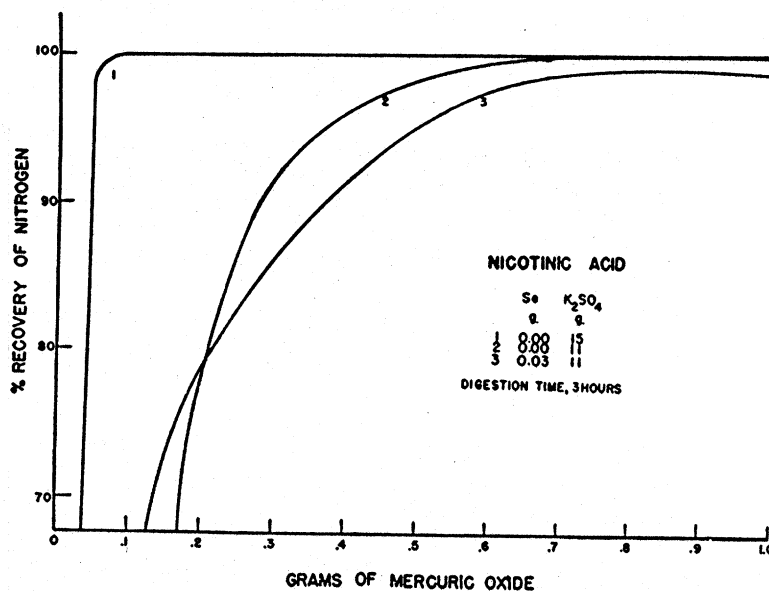


FIG. 6.—Recovery of nitrogen as affected by varying the amounts of mercuric oxide with different amounts of selenium and potassium sulfate.

For the smaller amount of potassium sulfate, theoretical recoveries were obtained only when more than 0.6 g. of mercuric oxide was used. The presence of selenium prevented the complete recovery of nitrogen with all amounts of mercuric oxide used.

Table 1 gives the results of the analyses of other refractory nitrogen compounds. With the digestion mixture consisting of 15 g. of potassium sulfate, 0.4 g. of mercuric oxide, and 25 ml. of sulfuric acid, and a digestion time of 3 hours, the amounts of nitrogen recovered were in close agreement with the theoretical values.

The preceding experiments showed that a simple digestion mixture of potassium sulfate, mercuric oxide, and sulfuric acid was sufficient for refractory compounds, but it was not certain what would happen to a less refractory material when digested for 3 hours or longer. Table 2 shows the recovery of nitrogen from S-benzyl thiuronium chloride with $\frac{1}{2}$ - to 6-hour digestion periods. Complete recovery of nitrogen was ob-

TABLE 1.—Per cent nitrogen found in several typical refractory nitrogenous compounds

refractory nitrogenous compounds			
	NITROGEN		
	FOUND BY ANALYSIS		THEORETICAL VALUE
	A	B	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Tryptophane	13.60	13.60	13.72
Histidine monohydrochloride	19.94	19.93	20.05
Lysine monohydrochloride	15.24	15.24	15.34
8-hydroxy quinoline	9.61	9.62	9.65
Nicotinic acid	11.38	11.40	11.38

TABLE 2.—Per cent nitrogen recovered from *S*-benzyl thiuronium chloride* using the potassium sulfate-mercuric oxide digestion mixture

DIGESTION TIME, HOURS	NITROGEN RECOVERED
	<i>per cent</i>
0.5	13.81
1	13.82
2	13.85
3	13.83
4	13.84
5	13.82
6	13.83

* Theoretical nitrogen value, 13.82.

tained after half an hour of digestion, and there was no appreciable loss up to 6 hours.

DISCUSSION

These experiments have demonstrated that the time of digestion and the amounts of potassium sulfate and of mercuric oxide used have an interrelated effect on the loss of nitrogen when selenium is present. This may be the reason that many have inaccurately defined the cause of incomplete recovery of nitrogen when selenium was used in the digestion mixture. Since selenium (1) may cause either incomplete recovery of nitrogen or a diminished rate of recovery, and (2) requires a specific time of digestion, its use cannot be recommended.

When selenium is omitted from the digestion mixture, the amount of potassium sulfate, mercuric oxide, and the time of digestion are not critical, insofar as loss of nitrogen is concerned. It was possible, therefore, to increase the amount of potassium sulfate so that complete recovery was obtained in 2 hours with no loss of nitrogen on prolonged digestion.

For the complete recovery of nitrogen from all kinds of materials which may contain refractory nitrogenous compounds, it is recommended as a general procedure that a digestion mixture consisting of 25 ml. of sulfuric acid, 15 g. of potassium sulfate, and 0.6 g. of mercuric oxide be used, with a digestion time of 3 hours. This method, which is a result of the studies reported here, is essentially the same as the A.O.A.C. method (12), and is further proof of its general applicability. If the 15 g. of potassium sulfate is carefully measured, 0.4 g. of mercuric oxide is more than sufficient, as shown in Figure 6. However, if less than 15 g. is added, as is often the case, 0.6 g. of mercuric oxide should be used.

In Figures 4 and 5 the curves for 9 and 11 g. of potassium sulfate, both with and without selenium, almost coincide, whereas the similar paired curves for 13 and 15 g. are markedly different. This peculiarity may explain why different analysts, using presumably the same Kjeldahl method, often obtain different results for refractory compounds. Since potassium sulfate is usually measured by volume instead of by weight, it would be possible for one analyst to add 13 and the other 11 g., and each think that he was following the procedure rigorously. Should both use a 4-hour digestion, one would obtain complete recovery and the other only 92 to 95% recovery.

The desirability of using a mercury compound as a catalyst is beyond question. The omission of mercuric oxide from the digestion mixture always causes incomplete recovery of nitrogen from nicotinic acid. With high concentrations of potassium sulfate, as little as 0.1 g. of mercuric oxide is sufficient.

SUMMARY

Complete recovery of nitrogen can be obtained from heterocyclic nitrogen ring compounds by the Kjeldahl method. The only catalysts required are mercuric oxide and potassium sulfate, used in the ratio of 0.6 to 15 g., with 25 ml. of sulfuric acid, and a digestion time of 3 hours. Because of the danger of loss of nitrogen when selenium is present, the use of selenium as an additional catalyst cannot be recommended.

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